

# MEASURING PROTEIN QUALITY IN HUMANS: A REVIEW AND PROPOSED METHOD<sup>1</sup>

V. R. YOUNG, W. M. RAND, and N. S. SCRIMSHAW, Department of Nutrition and Food Science, Massachusetts Institute of Technology, Cambridge, MA 02139

## ABSTRACT

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Various aspects of the determination of dietary protein quality in human subjects are reviewed. A brief account is given first of some of the factors that affect organ and whole body protein synthesis and breakdown, in order to provide a metabolic rationale on which to assess the various approaches which have been taken to determine protein quality in man. A short discussion of current knowledge concerning amino acid requirements and of factors that affect them and the requirement for protein is also considered in relation to the nutritional significance of dietary protein quality in subjects at various ages and under differing circumstances. Clinical methods for the evaluation of protein quality are reviewed and an analysis is presented of nitrogen (N)

balance data from a series of studies in young adult men receiving graded levels of intake of various test protein sources. The importance of multiple levels of test protein intake for critical estimation of protein quality is stressed. Analysis of the nitrogen balance data indicates that protein quality is estimated more precisely from an evaluation of the intersection of the N balance response curve with the line of N equilibrium as obtained with the test protein and compared with that of a reference protein, such as egg or milk protein. This approach is defined as the Relative Nitrogen Requirement (RNR). The RNRs for a soy protein isolate and whole ground wheat protein were 0.83 and 0.68, respectively, when studied in young adult men.

Ultimately, the quality of food proteins intended for direct human consumption should be evaluated by direct experiments in human subjects. We have recently reviewed various aspects of dietary protein quality for human nutrition (1-3). Some of the important issues to be considered in the clinical assessment of dietary protein quality will be discussed only briefly and the interested reader is referred to the more extensive reviews (1-6) for a detailed treatment of the areas which are discussed below.

Generally, protein sources of animal origin are capable of meeting the physiological requirements for amino acids at dietary intakes which are lower than those required if the diet contains single sources of plant protein foods. Therefore, an adequate knowledge of amino acid and nitrogen (N) requirements is the traditional basis upon which to judge the quality of a food protein or mixture of food proteins. Hence, it is worthwhile to discuss some aspects of body protein metabolism and the requirements for N and essential amino acids before giving an account of the approaches for assessing the nutritive value of food proteins in human subjects. The major focus of this review will be given to human studies and no attempt will be made here to review the subject matter below in relation to results obtained in experimental animals.

## BODY PROTEIN SYNTHESIS: RELATION TO SUBJECT AGE AND PROTEIN INTAKE

The most important quantitative function of dietary protein is to furnish substrate necessary for tissue and organ protein synthesis. Hence, a brief account

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of some of the factors which affect the status of organ and whole body protein synthesis and breakdown is relevant to an evaluation of protein quality in human nutrition.

The rate of whole body protein synthesis per unit of body weight declines with continued growth and development within mammalian species (7). A number of studies confirm that this process also occurs in man (8-11). Table I provides a summary of results for whole body protein synthesis, as reported by Picou and Taylor-Roberts (8) and from our studies (12-15) in neonates, young adults, and elderly subjects. These observations indicate that the intensity of whole body protein synthesis declines rapidly during the first year of life, with the rate in young adults being less than 20% of that determined in premature babies. Our limited data indicate that the rate of protein synthesis per unit of body weight shows only a small further decline in the elderly (14,15), which appears to be related to the probable loss of body cell mass with progressive aging (*e.g.*, refs. 16,17).

The significance of these changes in body protein synthesis for their relation to the requirements for dietary protein and to dietary protein quality cannot be judged precisely. However, it may be of interest to emphasize that when the values for whole body protein synthesis rates (Table I) are compared with recommended allowances for dietary protein for the various age groups, the changes in estimated protein needs parallel the decline in intensity of total body protein synthesis (Fig. 1). Thus, the rate of total body protein synthesis per g of protein allowance does not vary markedly with age, suggesting that the intake of good quality dietary protein necessary to support whole body protein synthesis in healthy subjects may be relatively constant for all age groups when expressed in this way.

Another observation arising from studies of the dynamic aspects of whole body protein metabolism is that the amount of protein synthesized per day, per unit body weight, is considerably greater than the protein intake required to meet the most generous estimates of dietary protein requirements. Therefore, the amino acids which are liberated during the normal course of tissue and organ

TABLE I  
Mean Rates of Whole Body Protein  
Synthesis in Humans at Various Ages<sup>a</sup>

Group	Age	Body Protein Synthesis g/kg body wt/day
Neonate	1-46 days	18.0
Infant	10-20 months	6.9 <sup>b</sup>
Young adult		
Male	20-25 years	3.3
Female	18-23 years	2.6
Elderly		
Male	68-72 years	2.9
Female	69-91 years	2.3

<sup>a</sup>Mean values. Based on data in refs. 12-15 and unpublished results.

<sup>b</sup>From Picou and Taylor-Roberts (8).

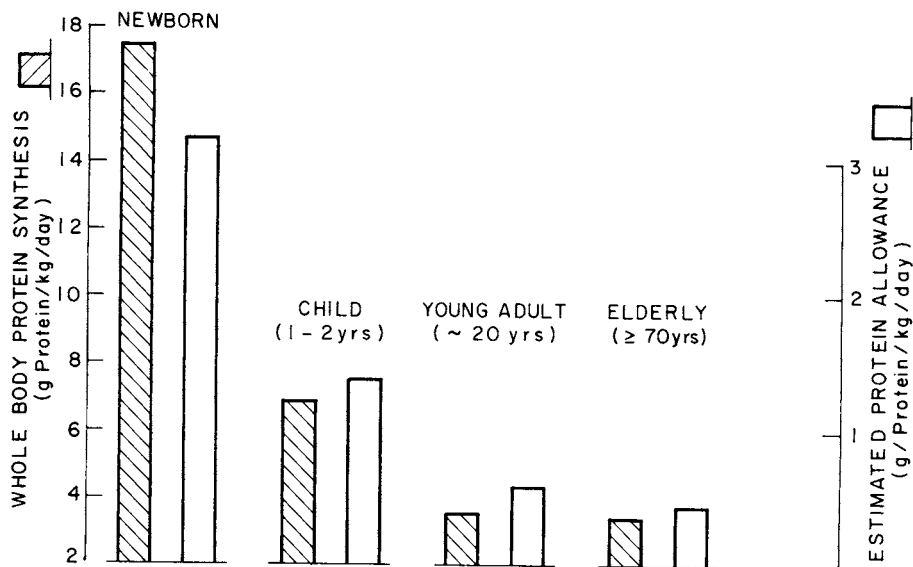


Fig. 1. Whole body protein synthesis in humans at various ages in relation to estimated dietary allowances for high quality protein. The latter are largely based on the allowances proposed by FAO/WHO (31).

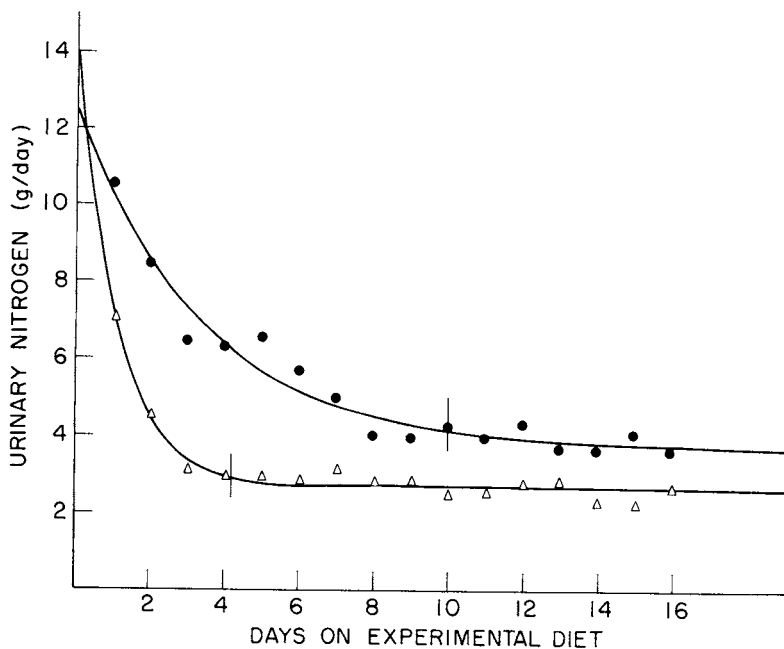


Fig. 2. Adaptive decrease in urinary nitrogen excretion in two young men receiving a protein-free diet. Taken from Rand *et al.* (55).

protein breakdown are extensively reutilized for protein synthesis.

The proportion of the released amino acids which are recycled, as well as the rates of synthesis and breakdown of whole body protein, will vary among individuals and may also change in response to changes in diet adequacy and the length of time a particular intake level is maintained. These aspects of body protein metabolism are also important in the evaluation of dietary protein quality.

As shown in Fig. 2, when a subject is placed on a reduced level of total protein intake, urinary N output decreases until a new steady state of N excretion is achieved. If protein intake is reduced below a minimum level, the subject will not maintain N equilibrium and a continued depletion of body protein will occur. Although data in human subjects are limited, they are sufficient to suggest the way in which body protein metabolism adjusts to changes in the level of protein intake.

Briefly, a low-protein diet, or one which is low in essential amino acids, results in a decrease in the content of essential amino acids in blood plasma (18,19). The plasma amino acid response depends on which particular essential amino acid is in short dietary supply (20). A phenylalanine-tyrosine-devoid diet does not change the level of plasma-free phenylalanine in young men during a 12-day

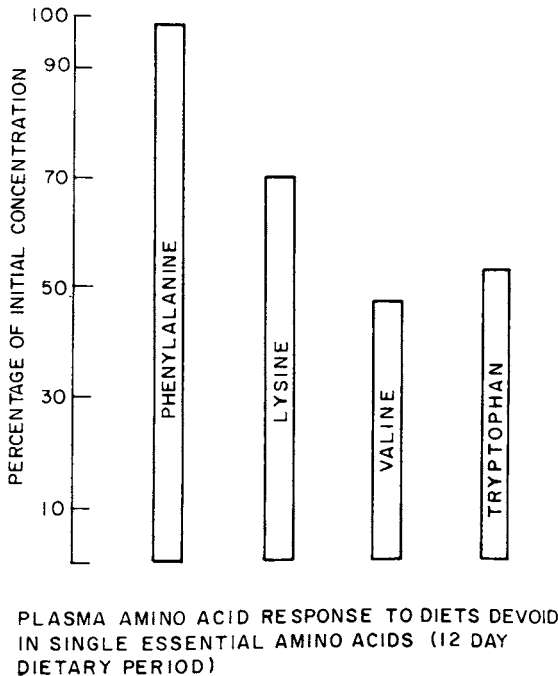


Fig. 3. Changes in the concentration of plasma free essential amino acid levels when young men receive diets devoid of these amino acids for 12 days. Taken from Young and Scrimshaw (3).

period, whereas a valine-free diet caused a significant decline in the concentration of plasma valine during the same period of time (Fig. 3) (20,21). The differential effects of specific dietary essential amino acid deficiencies on plasma amino acid levels reflect differences in the capacity of the individual metabolic pathways to adjust to alterations in the intake of specific amino acids.

Supporting such a conclusion are studies showing that the adult rat has a limited capacity to adapt to a deficient intake of threonine. In contrast, a dietary lysine deficiency brings mechanisms into play which result in an efficient conservation of this amino acid (22,23). The net effect of these differences in the metabolism of individual amino acids is that gross physiological responses, such as changes in body weight or changes in the efficiency of dietary N utilization, would be greater when a threonine-deficient diet is consumed, as compared to a diet which is limiting in lysine (*e.g.*, 23,25).

Differences in the metabolism of individual amino acids with dietary changes

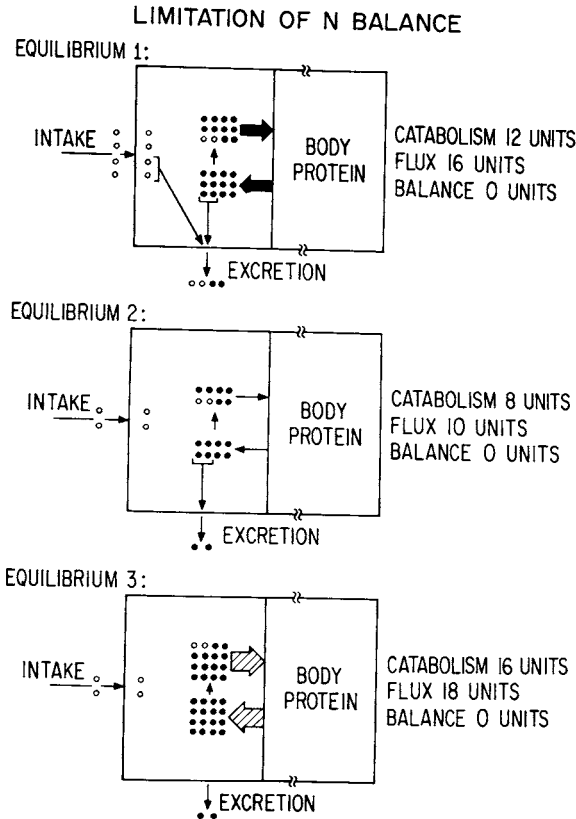


Fig. 4. A diagrammatic representation of two possible ways leading to a new equilibrium in N balance following reduced intake of dietary nitrogen. From Young and Scrimshaw (3).

raise complex problems for assessing the nutritional effects of various dietary proteins. Thus, the experimental conditions suitable for studying the nutritional value of proteins in which threonine is the first limiting amino acid are not necessarily the best conditions for exploring the quality of proteins which are limiting in lysine.

In addition to changes in the metabolism of individual amino acids, alterations in dietary protein intake have marked effects on the status of protein metabolism of individual tissues and organs. The changes are multiple, they differ among the various organs, and they are finally integrated into the total N economy of the intact organism; the total metabolic response of the organism is of importance in the context of the usual measures of dietary protein quality (e.g., 10,18).

Changes in whole body protein and N metabolism of human subjects have often been evaluated by the N balance technique. However, as already stated, a change in N intake may bring about an increase or decrease in the efficiency of N retention so that a new equilibrium is achieved in response to a decreased or increased N intake. Alternatively, a reduced intake of N (or amino acids) may result in a reduced rate of body protein breakdown, without a change in the efficiency of utilization of N ingested, but the net effect is also to achieve a new equilibrium in N balance (Fig. 4). Various mechanisms are possible as discussed

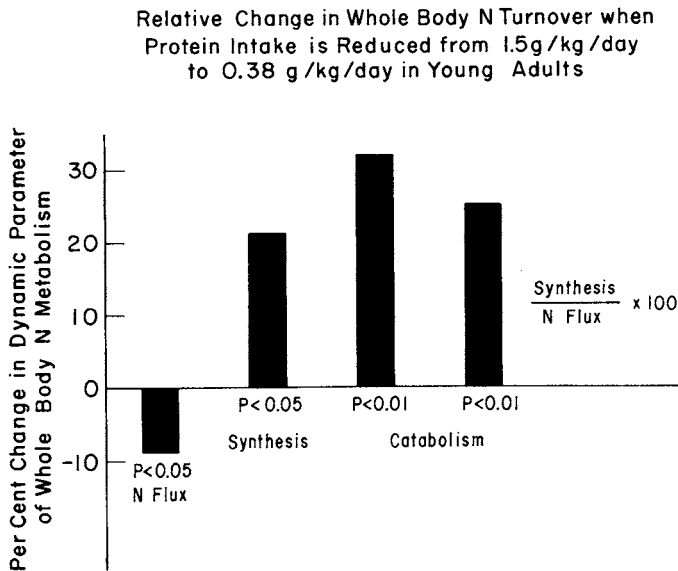


Fig. 5. Changes in body nitrogen flux and whole body protein synthesis and breakdown rates with reduced protein intake in young men. Drawn from results of Steffee *et al.* (12). The flux of nitrogen through the metabolic N pool and rates of whole body protein synthesis and breakdown were determined in six healthy young adults given a diet supplying 1.5 g high quality protein/kg/day and after two weeks of consuming 0.38 g egg protein/kg/day. The bars shown here represent the mean per cent increase or decrease in these parameters of whole body protein metabolism following adjustment of the subjects to the lower protein intake.

previously by us (3) and by Waterlow (26). Clearly N balance determinations alone do not provide an answer.

There are a few studies which allow a distinction to be made among these possibilities, the major limitation being the lack of adequate methodology. Studies in rats (27) suggest that a short-term adaptation to a low-protein diet is not accompanied by a decrease in total body N turnover, but by a more efficient utilization of N (amino acids) entering the metabolic pools. A similar conclusion may be drawn from the limited, short-term dietary studies in infants (8) and adults (12) (Fig. 5). However, the nature of the changes in body and organ N turnover with long-term ingestion of inadequate diets is not known for human subjects.

It can be seen from the above that amino acid and protein metabolism respond to alterations in the level of protein and amino acid intake via changes in tissue protein synthesis and breakdown, and by alterations in the efficiency of utilization and recycling of amino acids for protein synthesis. The responses help to explain why there are changes in the efficiency of utilization of dietary proteins when ingested at different levels of intake within the submaintenance-to-maintenance intake range. Figure 6 summarizes results obtained for the biological value (BV) of egg protein in a series of studies in young adults given egg protein at different intake levels. As can be seen, the BV, or retention of absorbed N, is high at low intake levels of test protein, but there is a significant fall in the calculated BV of egg as the test level of intake is increased and approaches the requirement for this protein source in young males. These observations provide a metabolic rationale for the proposition that the determination of dietary protein

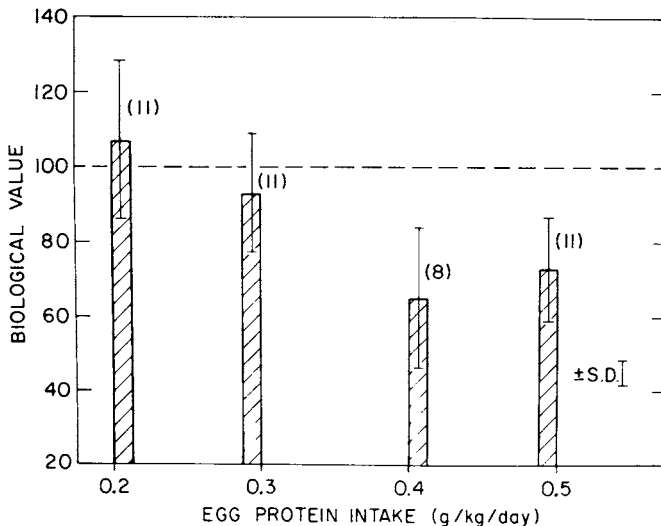


Fig. 6. Decrease in the calculated biological value of egg protein given to young men studied within the submaintenance-to-maintenance range of protein intake. From Scrimshaw and Young (28).

quality is best approached by measurement of a function of protein metabolism at graded levels of protein intake. This subject will be considered later in this review.

### AMINO ACID REQUIREMENTS

The essential amino acid content of proteins is an important determinant of the nutritional value of food protein sources. Thus, the essential amino acid requirements must also be understood and defined in order to determine the significance of protein quality in human nutrition. Some recent estimates of the essential amino acid needs of young infants and adults summarized in Table II do not reveal any marked differences in the pattern of amino acids required by the two age groups. This is even more evident when the lack of precision for each estimated amino acid requirement is recognized (29,30).

On the basis of current data (31), the absolute requirement for the essential amino acids declines more rapidly with age than does the need for total dietary N. It has been estimated that the infant requires approximately 38% of total N in the form of a balanced mixture of essential amino acids, but for the adult, this value is as low as 15% of the total N requirement (32). From these estimates, which indicate that the N and amino acid requirements change at different rates with age, Arroyave (33) developed a "protein quality index" to take these age-dependent changes into account. The summary of his calculations given in Table III implies that the nutritional quality of a protein must depend upon the age of the individual consuming it. If this concept were correct it would be of considerable nutritional importance because it suggests that dietary protein quality is of much greater significance in the infant and child than it is in the adult. Further, this implies that the determination of protein quality for infants and children must be carried out in the comparable age group.

However, the extent to which age affects the quality of a protein source is still

TABLE II  
Pattern of Essential Amino Acid  
Requirements in Infants and Adults<sup>a</sup>

Amino Acid	Relative to:			
	Valine		Lysine	
	Infant	Adult	Infant	Adult
Histidine	0.3	...	0.3	...
Isoleucine	0.8	1.0	0.7	0.8
Leucine	1.7	1.4	1.6	1.2
Lysine	1.0	1.2	1.0	1.0
S-Amino acids	0.6	1.3	0.6	1.1
Aromatic amino acids	1.3	1.4	1.2	1.2
Threonine	0.9	0.7	0.8	0.6
Tryptophan	0.2	0.2	0.2	0.3
Valine	1.0	1.0	0.9	0.8

<sup>a</sup>Taken from Young and Scrimshaw (3), based on FAO/WHO (31).



quite uncertain. Bressani (34) compiled a series of published results for the BV of various protein sources, as shown in Table IV. These data do not suggest a clear age-dependent trend in dietary protein quality. On the contrary, the figures tend to be similar for children and adults, particularly for the better quality proteins. However, a definitive conclusion concerning the importance of age as a variable in the assessment of protein quality should be based on more than BV and this measurement of protein quality is now recognized to be an inadequate index of protein nutritional value (3). It is noteworthy that the 1973 FAO/WHO Expert Committee (31) applied the same protein quality correction to all age groups in arriving at an estimation of the amount of poor quality proteins required to meet the protein needs of population groups.

### VARIATIONS IN PROTEIN AND AMINO ACID REQUIREMENTS

Before discussing clinical methods and approaches for the determination of protein quality, it is important also to take into account the variation in quantitative requirements of essential amino acids and total N among and within individuals. Variations in the requirements for protein and essential amino acids are due both to inherent genetic potential and to the modifying effect of

TABLE III  
Protein Quality Index<sup>a</sup> of Cow's Milk and Corn Proteins, in Relation to Age

	Cow's Milk	Corn
Infant	100	52
Adult	141	95

<sup>a</sup>Protein quality index: (Protein requirement for age ÷ Amount test protein to satisfy requirement for limiting amino acid) × 100. Arroyave (33).

TABLE IV  
Effect of Age on Biological Value of Proteins<sup>a</sup>

Protein	Subject Group	Protein Intake g/kg/day	Biological Value
Milk	Children	0.6	84
Milk	Adult	0.4	74
Egg	Children	0.6	97
Egg	Adult	0.3	89-96
Egg	Adult	0.2	94
Opaque-2 corn	Children	0.6	87
Opaque-2 corn	Adult	0.3	80
Common corn	Children	2.2	32
Common corn	Adult	...	57
Common corn	Adult	0.6	46

<sup>a</sup>Bressani (34).

environment on the expression of that potential. In addition, inborn errors of amino acid metabolism represent abnormal genetic variants with profound nutritional consequences for a few individuals (*e.g.*, ref. 35). For healthy persons, biological variation in the requirements is assumed to be normally distributed, but many factors can skew this distribution. Some of the biological factors which affect the requirements for protein (essential amino acids and N) are listed in Table V. The importance of these factors has been qualitatively demonstrated but information on their quantitative significance is meager. Before the practical significance of current estimates of protein and amino acid needs and of protein quality for various population groups can be judged fully, studies must be conducted in these groups to determine the effects of these factors on the utilization of differing protein sources.

### CLINICAL METHODS FOR EVALUATION OF PROTEIN QUALITY

Clinical methods for the evaluation of protein quality are based on the same principles applied to the corresponding animal assays, but require some modification for application to man. The principal procedures use as criteria either growth or N balance, alone or sometimes in combination with biochemical analyses of serum, proteins and amino acids, hemoglobin, blood urea N, and the urinary excretion of creatinine, sulphur compounds, and hydroxyproline.

#### *Some Used and Potential Criteria for Evaluation of Protein Quality in Man*

##### Growth

Weight

Height

Lean body mass

<sup>40</sup>K Whole body counting

Isotope dilution (<sup>3</sup>H<sub>2</sub>O; D<sub>2</sub>O)

Body density

Creatinine height index (CHI)

Hydroxyproline index (Hypro I)

##### Serum Levels

Proteins; albumin, enzymes

Free amino acids

Urea N

##### Nitrogen Balance

Nitrogen excretion

Sulphur excretion

N balance and growth are alternative criteria which are thought to give essentially similar results. Except in young infants, however, growth methods are too time-consuming and too subject to environmental influences to be convenient and are seldom used except for demonstrating the value of new protein foods designed for mass feeding of young children. A further important limitation to growth methods is that dietary protein cannot ethically be fed to growing children for long periods of time at the relatively low levels of intake required for detecting differences in protein quality. The N balance method has significant limitations (*e.g.*, 36,37), and requires sophisticated facilities and

personnel. However, it allows an evaluation of protein quality in a shorter time with fewer subjects than for growth studies.

In theory, a more refined growth criterion than overall increase in body weight or height would be estimation of the change in body protein or N. Currently, this approach is limited by the fact that methods for estimation of body protein content are indirect, relatively imprecise, and only available at a few research centers.

During recovery from protein malnutrition, serum albumin regeneration is slower with vegetable protein than with isonitrogenously fed milk (38). This has led to the suggestion that serum albumin regeneration might be used as a sensitive measure of protein quality (39,40). However, because the amino acid composition of serum albumin is unrepresentative of most other body proteins, its metabolism may not reflect the overall status of body protein metabolism (41), and is of doubtful value.

The majority of studies which have been conducted in humans for determining protein quality are based on direct or indirect measures by body N retention. The most widely used assays have used BV, net protein utilization (NPU), or apparent N retention as criteria. BV is NPU corrected for digestibility of the protein. In the standardized procedures for the estimation of BV and NPU, the test protein is given at a low and grossly inadequate intake level. The assumption is made that the nutritional value of a protein is constant throughout the submaintenance range, but Fig. 7 shows that the calculated BVs for egg and wheat gluten in young men are much lower when determined at close to maintenance levels of intake than at the lower levels of intake (42). These data indicate that the NPU and BV of a protein depend upon the level of intake in the test diet and other studies in humans support this conclusion (43,44). Similar observations were made in rats many years ago (see Ref. 4).

It is therefore difficult to draw unequivocal conclusions from most of the earlier studies on the comparative quality of protein sources for humans since the

**TABLE V**  
**Dietary, Host, and Environmental Factors that May Affect the**  
**Dietary Protein and Amino Acid Requirements of People**

Dietary Factors	Host Factors	Environmental Factors
Amino acid (Content and pattern)	Age Sex Genetic	Physical ( <i>i.e.</i> , temperature)
Presence (or absence) of other dietary constituents	Physiological states Growth Pregnancy Lactation	Biological ( <i>i.e.</i> , infectious agents) Social Dietary habits
Food processing	Aging Pathological states Trauma Neoplasia Other stress	Environmental sanitation Personal hygiene Physical activity

BV or NPU methods were used. As an example, the data reported by Hawley *et al.* (45) are summarized in Fig. 8. Although the BV of egg was high in these studies, the lower values for the other protein sources tested could be due in part to the higher level of protein intake used for evaluating these proteins, and not necessarily due to a less favorable essential amino acid content for meeting

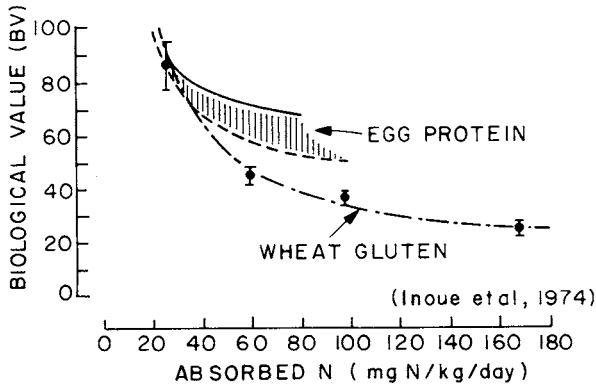


Fig. 7. Reduction in the biological value of egg and wheat proteins (gluten) given to young men. From Inoue *et al.* (42).

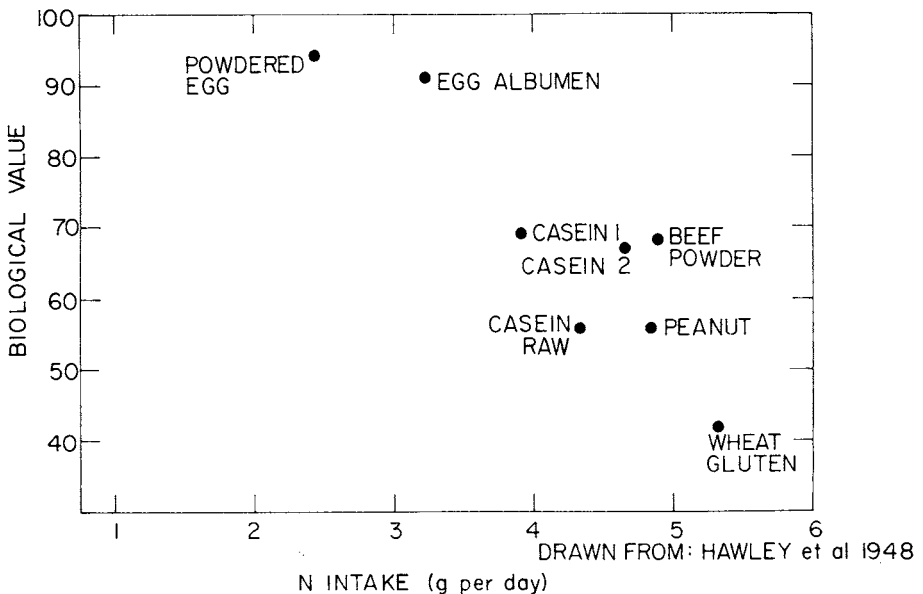


Fig. 8. Biological value of six protein sources, related to level of test protein intake. Drawn from data of Hawley *et al.* (45).

human requirements. Studies in children (46) have also shown an inverse relation between protein intake and protein value, again emphasizing the importance of considering the levels of protein intake in assessing the quality of proteins.

Clinical assays involving a single test protein level are inadequate, quantitative measures of protein quality. Useful comparative information on N utilization can be obtained in studies involving only one test protein intake level, but the limitations of the experimental design in relation to quantitative estimates of protein quality must be recognized. The preceding discussion indicates why. Assays involving more than one test protein level and, in particular, those which determine response to multiple doses of test protein, are necessary for the critical determination of dietary protein quality in human subjects, as well as in experimental animals (5,6,24).

Whether the assays are done at one level of protein intake or at multiple levels, the quantitative outcome is strongly influenced by caloric intake. It has long been recognized that at deficient levels of dietary calories some protein is used to meet energy needs. Recently (47-49), attention has been drawn to the fact that even a modest excess of dietary calories improves N utilization above that possible at caloric equilibrium. Most past N balance studies on human subjects have been done at generous levels of dietary energy and the data biased in the direction of higher protein values to some extent.

#### EVALUATION OF RESULTS OF N BALANCE MEASUREMENTS FOR PROTEIN QUALITY ESTIMATION

It should be clear from the foregoing that the evaluation of protein quality in humans is not a simple problem. This is not only because of changes which occur in body protein metabolism in response to altered dietary intakes and the difficulty of determining dietary energy requirements, but also because of the metabolic and nutritional variability of individuals. Individuals vary in their efficiency of utilization of a specific protein source and this utilization, even for the same individual, varies with time and circumstance. This raises practical difficulties because it means that relatively large numbers of subjects are required to define adequately a population response to a specific protein source. Most human studies of dietary protein quality compromise on small numbers of subjects.

Recognizing these limitations and problems, we present data obtained in a series of N balance experiments in young adult subjects studied at several different protein intake levels and involving three protein sources. We use them to illustrate alternative approaches to the determination of protein quality in human nutrition. Each approach begins by determining the N balance response curve, formalizing the relation between N (protein) intake and N balance in each study. The experimental methods and design of the studies with egg and soy proteins were comparable to those which followed in our earlier study with wheat protein (50).

Two different response criteria can be used to evaluate dietary protein quality from the N balance results (Fig. 9). The first involves an estimation of the efficiency with which dietary N is utilized. This is determined from the slope of the N balance response curve in the region of the maintenance N intake level. Secondly, protein quality can be estimated in relation to how well a given protein

source meets the requirement for protein and amino acids, *i.e.*, what minimum intake level of a given protein is required to maintain N equilibrium in adults or a predetermined rate of N retention in infants or children. In this case, the response criteria is the intersection of the N balance response curve with the line of N balance. Variability in requirements among individuals in their response can also be easily expressed. These two approaches in the determination of protein quality are independent and the comparative aspects of protein quality may be studied from the relation of the responses to a test protein to that obtained with a reference protein.

Three methods for estimating protein quality from these response criteria will be discussed (Fig. 9). The underlying assumption in these analyses, that the variables and errors involved follow a Gaussian distribution, is supported by data on the obligatory N losses of a large group of similar young adult male subjects (51). It is essential that each population be generally homogeneous and that it not consist of subgroups of "normal" and "abnormal" subjects.

#### N BALANCE METHODS FOR DETERMINING THE N BALANCE RESPONSE CURVE

##### Single Level of Test Protein Intake

In this method, NPU is calculated. It is assumed that the efficiency of dietary N utilization is constant over the range of N intake from zero to maintenance levels. The N balance response curve is assumed to be represented by a straight line which may be determined by the response to any two levels of N intake. For estimation of NPU, the zero intake level providing an estimate of obligatory N losses has been used as one of the levels. This latter is convenient because it is

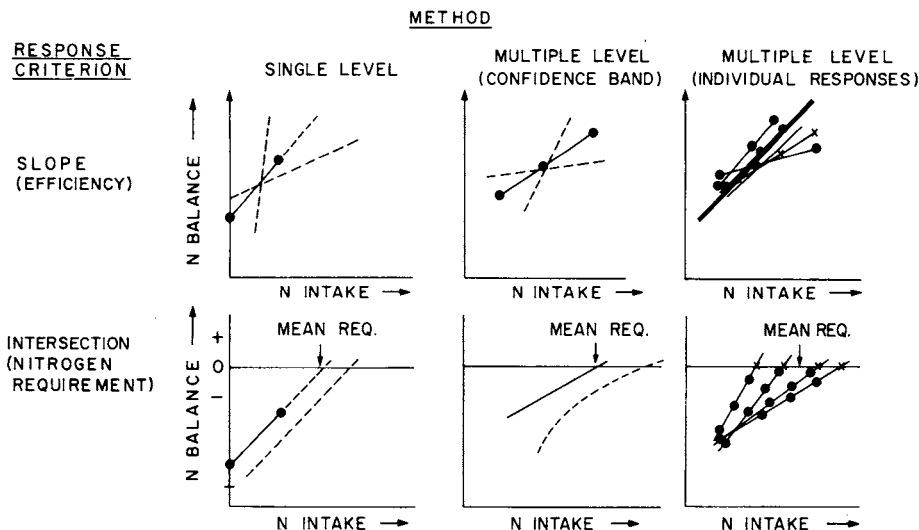


Fig. 9. A schematic depiction of two N balance response criteria and the three methods of estimation for the assessment of dietary protein quality in human subjects. The mean responses, their 95% (*slope*) or 97.5% (*intersection*) confidence limits, can be estimated.

independent of the dietary protein source and, therefore, needs to be determined only once for all protein sources.

For any particular dietary protein source, the N balance can in theory then be determined at a single level of test intake. In adult humans, the level used has often been about 0.3 kg/kg/day (1). The straight line through these two points is then the best estimate of the N balance response curve and the mean slope of the line describes dietary N utilization, or NPU.

The intersection of this response curve with the line of body N equilibrium provides an estimate of the mean protein requirement ( $PR_m$ ), while the standard deviation or requirement ( $PR_{sd}$ ) is assumed to be equivalent, in relative terms, to the standard deviation of N losses measured at the zero N intake. These two quantities can then be used to estimate PR 0.975, the 97.5% allowance for that population and protein source. The quality of a specific protein can, therefore, be assessed in terms of either NPU or protein requirement.

#### Multiple Levels

*Confidence Band (Pooled Data) Method.* This approach is based on the fact that the efficiency of dietary N utilization does not remain constant over the entire range from a zero to requirement levels of intake (42—44, 46). Thus, for this method, the N balance response curve is measured at multiple levels of N intake in the submaintenance - to - maintenance intake range. Standard least squares regression is used both to estimate the best straight line through the pooled N balance data from a number of subjects, and to calculate its variability. The slope of this line represents the efficiency of dietary N utilization. The standard error of the slope is used to determine confidence limits for this value.

Alternatively, the intersection of the N balance response curve with the line of body N equilibrium gives an estimate of the mean N requirement of the population. The standard error of the regression line is used to calculate confidence bands. The intersection of the upper 97.5% confidence band with the line representing total N losses gives an estimate of the intake of N which is adequate for nearly all of the population. The mean N requirement and the N intake sufficient for 97.5% of the population provide different possible measures of the quality of the protein fed.

*Individual Response Curve Method.* This consists of analyzing the N balance data for each individual by the confidence band method. For each subject, a linear regression line is calculated to estimate the individual N balance response. The mean efficiency of N utilization is the average value for the slopes of the individual regression lines and the estimated lower 95% confidence limit is obtained from the variability of the individual slopes. The intersection of these separate N-balance regression lines with the line of N equilibrium is used to calculate the mean N requirement for the group of individuals and the set of requirement estimates is then used to estimate an allowance sufficient to cover 97.5% of the population.

Table VI summarizes these various calculations giving N balance data for the groups of young men given egg, soy protein isolate, and whole ground wheat proteins.

*Comparative Protein Quality.* When the comparative nutritive value of a dietary protein is of interest, the ratio of the quality of the test protein with the quality of a reference protein (usually either milk or egg) can be calculated.

*Comparison of Protein Quality.* The obvious statistical test for difference between pairs of proteins is the "t" - test. This requires that the variances be equal, an assumption that can be tested by calculating an F statistic. If the variances are significantly different, then the Behrens-Fisher test (52) should be used. If differences among more than two proteins are to be compared, analysis of variance is required. Again, equality of variances should be checked by Bartlett's or Cochran's test (52). With these tests, the data given in Table VI may be used to explore further the various methods and response criteria as a basis for determining protein quality in human subjects.

First, the NPU of soy and whole ground wheat are essentially the same as for egg protein (Table VII). The mean N requirement for wheat, estimated from this method based on a single test level, did not differ from egg protein but soy was significantly lower in quality.

The comparative picture is different when the multiple-intake level method is used to estimate the efficiency of N utilization and the N requirement. In this case, the nutritive value of wheat protein is about 50% of that of egg protein, whereas there is no significant difference between the protein quality of soy and egg. On the other hand, if protein quality is determined from the estimated mean N requirement, soy protein was of lower quality than egg, and wheat still poorer. As shown in Table VII, evaluations of the N balance response curve for the

**TABLE VI**  
Estimates of the Nutritive Value of Egg, Soy Isolate and Whole Wheat Proteins as Determined from the Slope (Efficiency) and Intersection (N Requirement) of the N Balance Response Curve in Young Adult Men<sup>a</sup>

Protein Source	Method of Estimation	Slope (Efficiency)	Intersection (Requirement)
Egg	Single test level	0.63	81 <sup>b</sup>
	Multiple test level		
	Pooled data	0.50	88
	Individual responses	0.55	88
Soy isolate	Single test level	0.54	94
	Multiple test level		
	Pooled data	0.43	107
	Individual responses	0.43	107
Whole wheat	Single test level	0.65	78
	Multiple test level		
	Pooled data	0.26	130
	Individual responses	0.28	133

<sup>a</sup>Data based on N balance determinations in each of seven young men given egg, soy, or whole wheat proteins, as described in the text.

<sup>b</sup>Values are mg N/kg/day and represent N intake estimated to be just sufficient to maintain N balance. Integumental allowance of 5 mg/kg/day was made in estimating N balance.



various proteins by the pooled data and individual response curve methods give similar results.

A number of important conclusions can be drawn from these comparisons. Firstly, the NPU method does not adequately discriminate probable differences in the nutritive value of the three protein sources. NPU overestimates the quality of proteins, particularly those which are thought to be limiting in lysine. Our own results confirm the observations made in more extensive studies in rats (53).

Secondly, it is apparent that by pooling the N balance data for a group of subjects given graded intakes of test protein to estimate the N balance response curve, an estimate of protein quality is obtained which is comparable to that produced by analysis of the N balance response curves for each individual subject. Because the individual response curve method allows an opportunity to explore more critically the problems of variability within and among individuals, it is recommended that further work be carried out to realize the additional benefits to be gained by using this method whenever sample size is sufficient.

Finally, the data shown in Table VII suggest that there are differences in the ability of the two response criteria, slope and intersection with zero balance, to discriminate among dietary protein sources when tested at multiple intakes. Thus, the apparent nutritive value of the soy protein isolate was higher when the efficiency or slope criterion was used in comparison to the estimate of protein quality obtained by the requirement method. In the former case, the qualities of soy and egg were similar ( $P > 0.05$ ). However, in the latter case the protein quality of soy was about 80% ( $P < 0.05$ ) of that of egg protein. This suggests that the slope approach may be less discriminating than the intersection method. In this context, our findings lend support to the rat studies of McLaughlan and Keith (54) who observed that slopes of the response curves for proteins limiting in threonine were not different from those of the reference protein, lactalbumin. However, the intercepts of the response curves were displaced to the right, indicating that a higher intake of the threonine-limiting proteins was required to achieve a given response (*e.g.*, body weight gain) than when reference protein was fed.

Complete analysis of approaches to the estimation of protein quality requires

TABLE VII  
Comparative Protein Quality of Soy Protein Isolate and Whole Wheat  
Proteins for Each of Seven Young Men, Using Egg as Reference

Protein Source	Response Criterion <sup>a</sup>	Method of Estimation		
		Single Test Level <sup>b</sup>	Multiple Test Levels	
			Pooled data	Individual responses
Soy isolate	Efficiency	0.86	0.86	0.79
	Requirement	0.86*	0.83*	0.82*
Wheat	Efficiency	1.03	0.53*	0.52*
	Requirement	1.03	0.68*	0.66*

<sup>a</sup>Comparisons made of mean responses.

<sup>b</sup>Ratio of NPUs.

larger numbers of subjects and an assessment of the nutritional significance of the longer-term metabolic balance studies compared with short-term ones. However, we conclude from these data that the estimation of protein quality is best made by a comparative analysis of the estimated mean N (protein) requirements for various proteins. The latter can be determined from the individual N balance response curves in subjects given test and reference proteins at multiple levels within the range of submaintenance-to-near maintenance N intake. From our experience in studies of most food proteins in young adult men, the appropriate range lies between intakes of about 0.3 to a maximum of 0.7 g protein/kg/day. We propose that this measure be defined as the RELATIVE NITROGEN REQUIREMENT (RNR). Based on studies at four levels of protein intake, 1.5, 1.0, 0.75, and 0.5 g/kg/day, in young children, Viteri and Bressani (56) suggested use of the slope of the line describing N retained on N absorbed as the criterion for protein evaluation. However, they also indicated that comparison of levels of N intake necessary to maintain N equilibrium would be of practical value. Additional studies will be required to develop the best procedure for describing the RNRs of dietary protein sources and the variability of these values among different age and population groups.

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